Anti-inflammatory and anti-oxidative activities of *Stephania pierrei* extracts
ฤทธิ์ต้านการอักเสบและต้านอนุมูลอิสระของสารสกัดบัวบกป่า

Sirilak Kamonwannasit[1], Bung-on Prajanban[2], Pakarang Srimee[3], Agarat Kamcharoen[1]*
[1]Division of Agro-Industrial Product Development, Faculty of Agricultural Technology, Burapha University, Sa Kaeo 27160
[2] Division of Agricultural Innovation, Faculty of Agricultural Technology, Burapha University, Sa Kaeo 27160
[3]Faculty of Physical Therapy, Srinakharinwirot University, Nakhonnayok 26120
ศิริลักษณ์ กมลวรรณสิทธิ์[1], บังอร ประจันบาล[2], ปะการัง ศรีมี[3], เอกรัฐ คำเจริญ[1]*
[1]สาขาวิชาพัฒนาผลิตภัณฑ์อุตสาหกรรมเกษตร คณะเทคโนโลยีการเกษตร มหาวิทยาลัยบูรพา สระแก้ว 27160
[2]สาขาวิชานวัตกรรมเกษตร คณะเทคโนโลยีการเกษตร มหาวิทยาลัยบูรพา สระแก้ว 27160
[3]คณะกายภาพบำบัด มหาวิทยาลัยศรีนครินทรวิโรฒ นครนายก 26120
*Email : agratk@go.buu.ac.th

Abstract

A tuber of *Stephania pierrei* Diels (Menispermaceae) has been used as a traditional medicine for edema, migraine, and heart related to inflammation. The objective of this study was to determine the anti-oxidative and anti-inflammatory activities of *S. pierrei* tuber extracts including hot water extract (SH) and ethanolic extract (SE). Hydroxyl radical and superoxide radical scavenging activities were used to analyze their anti-oxidative activities Reverse Transcriptase-polymerase chain reaction (RT-PCR) was used to investigate the gene expression of pro-inflammatory mediators including IL-1β, COX-2, TNF-α, IL-6, and iNOS compared with a housekeeping gene, β-actin. In addition, the cytotoxicity of extracts against RAW264.7 macrophages was also determined by using an MTT assay.. The results showed that both SE and SH showed high anti-oxidative activity. The SE showed slightly stronger superoxide radical scavenging activity than SH. For cytotoxic effect, SE showed higher cytotoxicity than SH with IC_{50} of 96.14±1.79 μg/ml and 191.80±6.08 μg/ml, respectively. Both extracts as well as the standard compounds, significantly suppressed the gene expression of all pro-inflammatory mediators. The SE effectively suppressed COX-2 gene expression to the same degree as the SH (IC_{50} of 50.85±2.15 μg/ml and 56.54±0.97 μg/ml, respectively). Moreover, the SE showed a stronger inhibitory effect than the SH on the gene expression, especially IL-1β, IL-6 and TNF-α genes. These results indicated that *S. pierrei* tuber extracts might decrease inflammation via suppression of pro-inflammatory mediator gene expression and their anti-oxidative activity. Therefore, *S. pierrei* tubers can be a good candidate for the development of anti-inflammatory health products.

Keywords : *Stephania pierrei* Diels, Anti-oxidative activity, Anti-inflammatory activity, Cytotoxicity, Pro-inflammatory mediators

12 พฤศจิกายน 2564 / 11 มีนาคม 2565 / 30 มิถุนายน 2565
12 November 2021 / 11 March 2022 / 30 June 2022

บทคัดย่อ

ส่วนหัวใต้ดินของบัวบกป่านั้นถูกนำมาใช้ในการทางแพทย์พื้นบ้านใช้ในการรักษาโรคเกี่ยวกับการบวมน้ำ ไมเกรน โรคหัวใจที่เกี่ยวข้องกับการอักเสบ วัตถุประสงค์ของการศึกษานี้เพื่อศึกษาการต้านอนุมูลอิสระและการต้านการอักเสบของสารสกัดจากส่วนหัวใต้ดินของบัวบกป่าด้วยน้ำ (SH) และสกัดด้วยเอทานอล (SE) ด้วยการวัดอนุมูลอิสระไฮดรอกซิลและซุปเปอร์ออกไซด์ การตรวจสอบการแสดงออกของยีนของสารสื่อกลางการอักเสบ ได้แก่ IL-1β, COX2, TNF-α, IL-6 และ iNOS เปรียบเทียบกับ β-actin ด้วยวิธี Reverse transcription polymerase chain (RT-PCR) ตรวจสอบความเป็นพิษต่อเซลล์ของสารสกัดต่อเซลล์มาโครฟาส์ก RAW 264.7 ด้วยวิธี MTT จากการศึกษาพบว่า ทั้งสารสกัด SE และ SH มีฤทธิ์ต้านอนุมูลอิสระสูง สารสกัด SE มีฤทธิ์ต้านอนุมูลอิสระซุปเปอร์ออกไซด์ได้ดีกว่าสารสกัด SH เล็กน้อย การศึกษาของความเป็นพิษของสารสกัด พบว่า สารสกัด SE มีผลการทดสอบความเป็นพิษที่สูงกว่าสารสกัด SH ซึ่งมีค่า IC_{50} เท่ากับ 96.14±1.79 ไมโครกรัมต่อมิลลิลิตร และ 191.80±6.08 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ สารสกัดทั้งสองชนิด (SE และ SH) และสารประกอบมาตรฐานสามารถยับยั้งการแสดงออกของยีนของสารสื่อกลางการอักเสบทั้งหมดอย่างมีนัยสำคัญ โดยสารสกัด SE มีผลต่อการยับยั้งการแสดงออกของ COX-2 ได้ในระดับเดียวกับสารสกัด SH (IC_{50} เท่ากับ 50.85±2.15 ไมโครกรัมต่อมิลลิกรัม และ 56.54±0.97 ไมโครกรัมต่อมิลลิกรัม ตามลำดับ) นอกจากนี้ สารสกัด SE แสดงให้เห็นว่ามีผลการยับยั้งการแสดงออกของยีน IL-1β, IL-6 และ TNF-α ได้ดีกว่าสารสกัด SH จากผลการทดลองที่ได้แสดงให้เห็นว่า สารสกัดส่วนหัวใต้ดินของบัวบกป่าอาจมีผลลดการอักเสบได้โดยผ่านทางการยับยั้งการแสดงออกของยีนของสารสื่อกลางการอักเสบ และฤทธิ์ต้านอนุมูลอิสระได้ ดังนั้น ส่วนหัวใต้ดินของบัวบกป่านั้นเป็นอีกตัวเลือกหนึ่งที่เหมาะต่อการนำไปทำผลิตภัณฑ์เกี่ยวกับการต้านการอักเสบ

คำสำคัญ : บัวบกป่า ฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ต้านการอักเสบ ความเป็นพิษต่อเซลล์ สารสื่อกลางการอักเสบ

Introduction

Inflammation is one of the host defense mechanisms and conserved processes for eliminating harmful and foreign stimuli and promoting the repairing and recovery processes. However, this immune response may create adverse such as heat, redness, swelling, pain, chronic inflammatory diseases, or illnesses. Unregulated inflammation plays an important role in chronic disease development. Chronic inflammation is an important factor that can cause several chronic diseases such as cancer, atherosclerosis, rheumatoid arthritis, diabetes mellitus, Alzheimer's disease, and ulcerative colitis. In inflammatory responses, the immune and stromal cells secrete several pro-inflammatory mediators such as tumor necrosis factor (TNF-α), interleukin 6 (IL-6), interleukin 1β (IL-1β), cyclooxygenase-2 (COX-2) and iNOS.[1,2] Suppressive effects of anti-inflammatory agents on the pro-inflammatory mediator production can help to prevent and treat the inflammation.[3,4]

Stephania pierrei Diels (syn. *Stephania erecta* Craib) belongs to the family Menispermaceae. Its tuber has been traditionally used as the herbal medicine for the treatment of body edema, migraine, and heart disease.[5] These diseases and symptoms are related to inflammatory responses.[6,7] The *S. pierrei* extract was reported to have acetylcholinesterase (AChE) inhibitory activity with 89% inhibition at 0.1 mg/ml and IC_{50} of 6 µg/ml.[8] AChE inhibitors are well-known drug for Alzheimer's disease which is a chronic inflammatory disease.[7] In our previous

studies, we reported anti-oxidative, anti-fungal, and anti-bacterial activities of *S. pierrei* tuber hot water extract.[9,10] This extract was also found to contain a cepharanthine, an alkaloid in the plant's tubers. The cepharanthine is one of the medicine for the treatment of Alzheimer's disease.[11] Moreover, the *S. pierrei* extract was reported to show anti-cancer and anti-malarial activities related to the alkaloid content in the extract.[12] It is possible that the *S. pierrei* tuber extract may have anti-inflammatory activity leading to the effects used as an herbal medicine. However, there was no study on the anti-inflammatory activity of the *S. pierrei* extract. Therefore, the objective of this study was to investigate the anti-oxidative and anti-inflammatory activities of *S. pierrei* tuber extracts comparing hot water and ethanolic extracts.

Methodology

Plant extract preparation Tubers of *S. pierrei* were collected from Burapha University Sa Kaeo Campus, Wattana Nakorn District, Sa Kaeo Province. The plant was identified by a botanist, Dr. Chakkrapong Rattamanee. A plant specimen has been kept at the Faculty of Agricultural Technology, Burapha University Sa Kaeo Campus and the voucher specimen number is AgriTech-002. The tubers were sliced and then dried using a hot air oven at 50°C for 3 days. The dried pieces were mashed. The dried powder was divided into 2 parts. One part was extracted by using distilled water (400 ml) at 80°C for 1 h. Another part was extracted by using a continuous extraction method in a Soxhlet apparatus and 95% ethanol was used as a solvent. The extracted liquid parts were filtered, concentrated, and kept at 4°C.[13] The extract from hot water extraction was called *S. pierrei* hot water extract (SH) and ethanolic extraction was called *S. pierrei* ethanolic extract (SE).

Determination of anti-oxidative activity

Hydroxyl radical scavenging activity

The determination of hydroxyl radical scavenging activity was modified from a previous study.[14] Briefly, the extract was added with 200 µl of 2.8 mM deoxyribose, 220 µl of 10 mM potassium phosphate (pH 7.4), 380 µl of 2.8 mM H_2O_2, 200 µl of 25 mM $FeCl_3$, and 200 µl of 100 mM nitrilotriacetic acid (NTA). The mixture was incubated at 37°C for 1 h and added with 1 ml of 1% thiobarbituric acid (TBA) and 1 ml of 2.8% trichloroacetic acid (TCA). The mixture was incubated in a water bath at 100°C for 20 min. After cooling down, the mixture has measured the absorption at 532 nm and a Trolox was used as a positive control.

Superoxide radical scavenging activity

The determination method of superoxide radical scavenging activity was modified from Nishikimi et al.[15]. Briefly, the extract was added with 0.5 ml of 2.52 µM nitroblue tetrazolium (NBT) in 0.1 M phosphate buffer (pH 7.4) and with 0.5 ml of 624 µM nicotinamide adenine dinucleotide (NADH) in 0.1 M phosphate buffer (pH 7.4) and with 0.5 ml of 120 µM phenazine methiosulphate (PMS) in 0.1 M phosphate buffer (pH 7.4). The mixture was placed at room temperature for 14 min. The absorption was measured at 560 nm. The results were calculated for % inhibition and then expressed as 50% inhibitory concentration (IC_{50}) by comparing with the standard compound, gallic acid.

Determination of anti-inflammatory activity

Cytotoxicity test

Murine macrophages (RAW264.7) (1×10^4 cells/well) were cultured in Dulbecco's Modified Eagle Medium supplemented with 1% penicillin-streptomycin solution and 10% fetal bovine serum under 5% CO_2 at 37°C for 24 h.

The cells were then added with various concentrations of the diluted extract. After 24 h incubation, the culture media was removed and then analyzed cell viability by using an MTT assay.[16]

Pro-inflammatory mediator gene expression: The gene expression determination was analyzed using the reverse transcriptase polymerase chain reaction (RT-PCR) method. The RAW264.7 cells (1×10^6 cells/well) were cultured in a 12 well plate and incubated for 24 h. The culture media was removed. The cells were added with the selected concentration of extract and then incubated for 22 h. To induce the pro-inflammatory mediator gene expression, the cells were added with 1 µg/ml *Escherichia coli* lipopolysaccharides (LPS) and then incubated for 2 h. The cells were harvested and extracted for whole-cell RNA by using an RNA extraction kit (GE Healthcare, UK). The RNA was analyzed by using UV spectrophotometer at 260 and 280 nm and calculated for total RNA concentration and RNA quality. The RNA (40 ng) was reversed to cDNA by using reverse transcriptase from Omiscript RT Kit (QIAGEN, Germany). The cDNA (3 µl) was amplified by using the specific primers for β-actin, IL-1β, COX-2, TNF-α, IL-6, and iNOS (Table 1) and using a thermal cycler PCR (AB Applied Biosystems GeneAmp PCR system 2400). Each gene was amplified for 27 cycles and the amplifying condition was shown in Table 2. The PCR products were confirmed by using electrophoresis and visualized by using Novel Juice staining. The density of the PCR products was measured by using Gel Documentation and Analysis system machine. The results of gene expression were expressed as the relative mRNA expression level with β-actin. The inhibitory activity of extracts on each gene was expressed as IC_{50} by comparing with the gene expression level of unstimulated cells.

Table 1 Oligonucleotide primers used in RT-PCR

Genes	Primers	Sequences (5′-3′)	Product size (bp)
β–actin[1]	Forward	TCATGAAGTGTGACGTTGACATCCGT	285
	Reverse	CCTAGAAGCATTTGCGGTGCACGATG	
IL-1β[2]	Forward	CAGGATGAGGACATGAGCACC	447
	Reverse	CTCTGCAGACTCAAACTCCAC	
COX-2[1]	Forward	GGAGAGACTATCAAGATAGT	861
	Reverse	ATGGTCAGTAGACTTTTACA	
TNF-α[1]	Forward	ATGAGCACAGAAAGCATGATC	276
	Reverse	TACAGGCTTGTCACTCGAATT	
IL-6[2]	Forward	CATCCAGTTGCCTTCTTGGGA	463
	Reverse	GCATTGGAAATTGGGGTAGGAAG	
iNOS[1]	Forward	AATGGCAACATCAGGTCGGCCATCACT	454
	Reverse	GCTGTGTGTCACAGAAGTCTCGAACTC	

Note: (1) Won et al.[17] and (2) Sugawara et al.[18]

Table 2 The amplifying condition used in PCR

Genes	Denaturation/ RT Inactivation	Cycles			Final Extension	Hold
		Denaturation	Annealing	Extension		
β-actin	95°C, 2 min	94°C, 1 min	60°C, 1 min	72°C, 1 min	72°C, 10 min	4°C
IL-1β	94°C, 2 min	94°C, 45 s	60°C, 45 s	72°C, 1 min		
COX-2	95°C, 2 min	94°C, 1 min	60°C, 1 min	72°C, 1 min		
TNF-α	94°C, 2 min	94°C, 15 s	60°C, 1 min	72°C, 1 min		
IL-6	94°C, 2 min	94°C, 15 s	60°C, 1 min	72°C, 1 min		

Statistical analysis

The results in this study were performed in triplicate and represented in mean ± S.D. Significant difference was *P* value < 0.05. One-Way ANOVA and Post HOC multiple comparisons (LSD) were used for statistical analysis by using SPSS.

Results and Discussion

Anti-oxidative activity

In our previous studies, we reported the anti-oxidative of *S. pierrei* hot water extract. The *S. pierrei* extract contained alkaloids, triterpenoids, tannins and polyphenols.[9] We also found the cepharanthine content of 33.68 mg/g extract. In addition, the ABTS, DPPH and FRAP assays were also used but we found that *S. pierrei* hot water extract showed low effectiveness on anti-oxidative activity.[10] This low activity might be due to the difference in solvent used in the extraction. In the present study, different anti-oxidative assays and extraction solvents were used. The SE showed slightly stronger superoxide radical scavenging activity than SH, whereas SH showed slightly higher hydroxyl radical scavenging activity than SE (Table 3). This high anti-oxidative activity of SH in the present study might be the result of water temperature during extraction. In our previous studies, we used boiling water for extraction[9,10], whereas we used water temperature of 80°C for extraction in this present study. These results indicated that some bioactive constituents might be degraded during high-temperature extraction resulting in low anti-oxidative activity in our previous studies. The extraction temperature, time and solvent type have a significant effect on the phenolic content yield, antioxidant capacity and biological activity.[19,20] However, both SH and SE in the present study showed high anti-oxidative activity and approximately 10 times lower activity than the standard compounds (Table 3). This high antioxidant of the extract might directly affect their anti-inflammatory properties.

Table 3 Anti-oxidative activities of *S. pierrei* extracts

Standard / Extract	Superoxide radical scavenging activity (IC_{50} mg/ml)	Hydroxyl radical scavenging activity (IC_{50} mg/ml)
Gallic	0.19±0.01[a]	-
Trolox	-	1.28±0.04[a]
SH	2.19±0.05[c]	12.8±0.08[b]
SE	1.79±0.01[b]	13.9±0.12[c]

[a] Different letter on the right side of the data within the same column indicated significant differences (n=3).

Cytotoxicity of extracts

After 24 h treatment, the extracts showed high cytotoxic effect. SE showed stronger cytotoxicity than SH with IC_{50} of 96.14±1.79 μg/ml and 191.80±6.08 μg/ml, respectively (Figure 1). This cytotoxicity might be the effect of the alkaloids in the extracts. Likhitwitayawuid et al.[12] reported the alkaloid content and the isolates from the *S. pierrei* extract and also showed that their cytotoxicity against cancer cells was mainly due to the presence of aporphine alkaloids. To prevent the cytotoxic effect on the tested cells, therefore, the extract concentrations between 25 to 100 μg/ml were selected for further experiments.

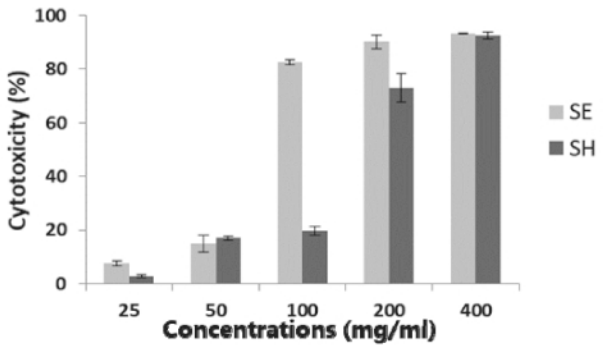

Figure 1 The cytotoxic effect of SH and SE against RAW264.7 cells (n=3)

Effect of *S. pierrei* extracts on pro-inflammatory mediator gene expression

In figure 2A, after LPS treatment the COX-2 gene was expressed in LPS-treated control cells (Lane 4) higher than the expression in unstimulated cells (Lane 1). The SE and SH at concentration of 25 to 100 μg/ml inhibited COX-2 gene expression in dose-dependent manner, and also a significant difference to LPS-treated control cells (Lane 4). SE could suppress the expression of COX-2 gene greater than SH with IC_{50} of 50.85±2.15 μg/ml and 56.54±0.97 μg/ml, respectively (Figure 3). In addition, SE also suppressed COX-2 gene expression at the same level as Indometacin, a positive compound (Lane 11).

In figure 2B, after treatment with LPS the expression of IL-1β in RAW264.7 cells was higher (Lane 4) than that of the unstimulated cell (Lane 1). The SE and SH at the concentration of 25 to 100 μg/ml inhibited the expression of IL-1β gene in dose dependent manner and showed a significant difference when compared with LPS-treated cells (Lane 4). The SE showed a stronger inhibitory effect on IL-1β gene expression than that of SH with IC_{50} of 60.50±3.26 μg/ml and 95.16±10.44 μg/ml, respectively (Figure 3). Moreover, SE could inhibit IL-1β expression at the same level as Indometacin (Lane 11). Interestingly, the 100 μg/ml SE could suppress the expression of the IL-1β gene to the same expression level of unstimulated cells (Lane 1).

In figure 2C, after treatment with LPS the expression of IL-6 in RAW264.7 cells was higher (Lane 4) than that of the unstimulated cell (Lane 1). The SE and SH at the concentration of 25 to 100 μg/ml decreased the expression of IL-6 gene in dose dependent manner and showed a significant difference when compared with LPS-treated cells (Lane 4). The SE showed a stronger inhibitory effect on IL-6 gene expression than that of SH with IC_{50} of 50.80±7.73 μg/ml and 84.79±13.88 μg/ml, respectively (Figure 3). Moreover, SE could inhibit IL-6 expression in the same level as Indometacin (Lane 11).

In figure 2D, after treatment with LPS the expression of iNOS in RAW264.7 cells was higher (Lane 4) than that of the unstimulated cell (Lane 1). The SE and SH at the concentration of 25 to 100 μg/ml suppressed the expression of iNOS gene in dose dependent manner and showed a significant difference when compared with LPS-treated cells (Lane 4). The SE showed an inhibitory effect on iNOS gene expression better than SH. The IC_{50} of SE and SH were 48.58±5.61 μg/ml and 61.25±3.94 μg/ml, respectively (Figure 3). In addition, SE could inhibit iNOS expression at the same level as Aminoguanidine, a positive compound (Lane 11).

In figure 2E, after treatment with LPS the expression of TNF-α in RAW264.7 cells was higher (Lane 4) than that of the unstimulated cell (Lane 1). The SE and SH at the concentration of 25 to 100 μg/ml reduced the expression of TNF-α gene in dose dependent manner and showed a significant difference when compared with LPS-treated cells (Lane 4). The SE showed an inhibitory effect on TNF-α gene expression better than SH with IC_{50} of 80.62±0.31 μg/ml and 94.86±13.37 μg/ml, respectively (Figure 3). In addition, SE could inhibit TNF-α expression in the same level as Indometacin (Lane 11).

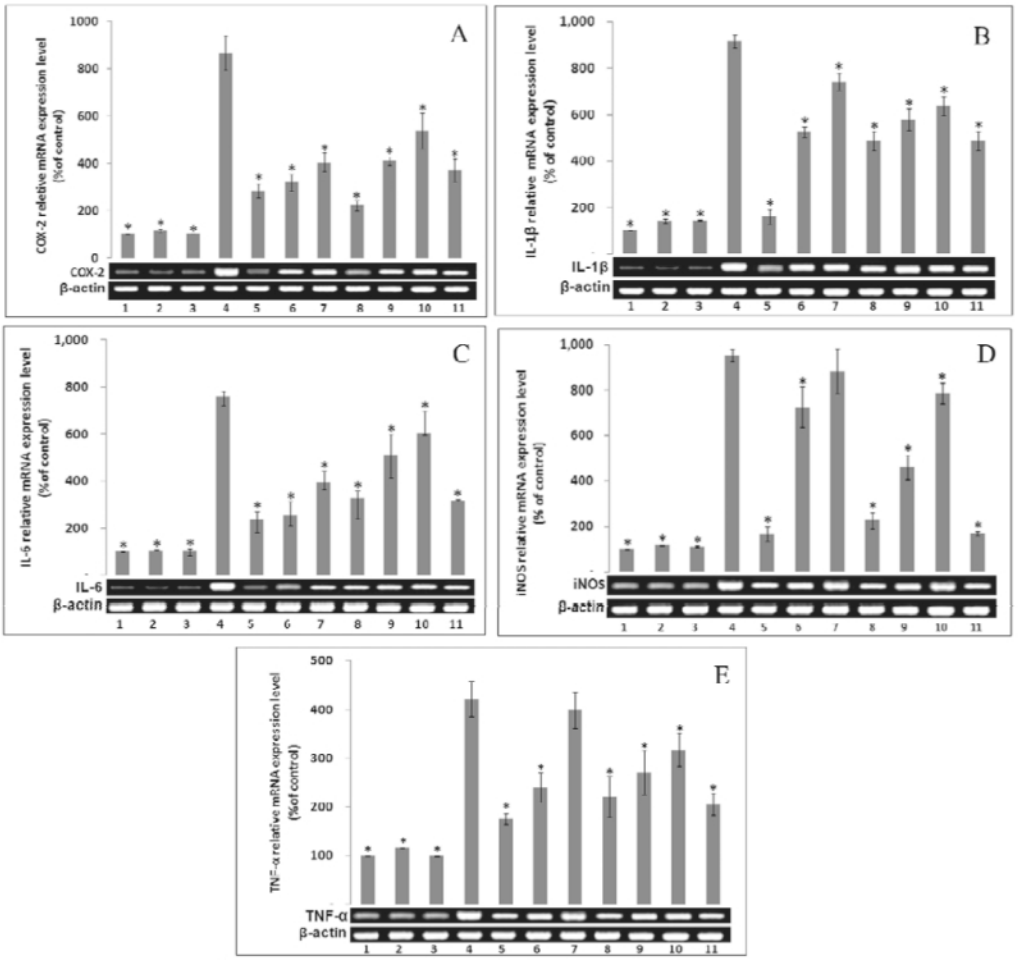

Figure 2 Effect of SE and SH on pro-inflammatory gene expression. A) COX-2, B) IL-1β, C) IL-6, D) iNOS and E) TNF-α. Lane 1 = Unstimulated cells, lane 2 = 100 μg/ml SE-treated cells, lane 3 = 100 μg/ml SH-treated cells, lane 4 = LPS-treated control cells, lane 5 = 100 μg/ml SE + LPS-treated cells, lane 6 = 50 μg/ml SE + LPS-treated cells, lane 7 = 25 μg/ml SE + LPS-treated cells, lane 8 = 100 μg/ml SH + LPS-treated cells, lane 9 = 50 μg/ml SH + LPS-treated cells, lane 10 = 25 μg/ml SH +LPS-treated cells, and lane 11 = 50 μg/ml Indometacin + LPS-treated cells (or 50 μg/ml Aminoguanidine + LPS-treated cells in the case of iNOS). * represents significant difference when compared with lane 4 LPS-treated cells ($P < 0.05$). Data are means of triplicates.

The plant in genus *Stephania* has been used to treat several illnesses such as dysentery, pyrexia, tuberculosis, diarrhea, dyspepsia, urinary tract diseases, abdominal ills, asthma, ascariasis, dysmenorrhea, indigestion, wounds, headache, sore-breasts and leprosy.[21] The *S. pierrei* has also been traditionally used for the treatment of body edema, migraine, and heart disease.[5] This traditional use may be related to their anti-inflammatory activity. The plant in genus *Stephania* contained over 150 alkaloids together with flavonoids, lignans, steroids, terpenoids and coumarins and these constituents play an important role in their biological activity such as anti-oxidative

activity, anti-malarial activity, anti-microbial activity, anti-viral activity, anti-cancer activity, anti-inflammatory activity, analgesic activity, and immunomodulating activity.[21] In our previous study, the *S. pierrei* extract was reported to have triterpenoids, tannins, polyphenols and alkaloids, especially cepharanthine.[9] Cepharanthine is an alkaloid and isolated from plants of the genus *Stephania*. It is generally abundant in the tubers of the plant. This cepharanthine has been used as a drug that showed several biological activities, including antioxidant, anti-inflammatory activity and potential activity in Alzheimer's disease.[11] Therefore, it is possible that cepharanthine may play a role in anti-inflammatory activity in this present study and in their traditional use.

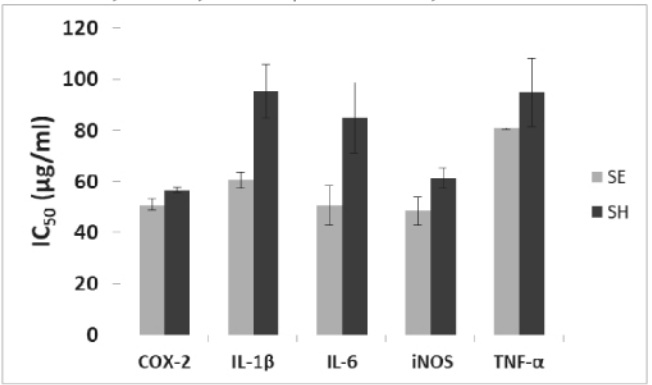

Figure 3 Comparison of inhibitory effect between SE and SH on COX-2, IL-1β, IL-6, iNOS and TNF-α gene expression

Conclusion

The extracts of *S. pierrei* in this study showed strong anti-oxidative activity. The extracts, especially ethanolic extract showed strong anti-inflammatory activities by suppressing the pro-inflammatory mediator gene expression, including IL-1β, IL-6, TNF-α, COX-2 and iNOS. Therefore, *S. pierrei* has the potential for further development of natural health products.

Acknowledgement

We would like to thank a botanist, Dr. Chakkrapong Rattamanee, for identifying the plant and making a plant specimen.

References

1. Culig Z. Cytokine disbalance in common human cancers. Biochim Biophys Acta 2011;1813:308-14.
2. Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso1 MA. Chronic inflammation and cytokines in the tumor microenvironment. J. Immunol. Res 2014;(149185):1-19.
3. Liu CH, Abrams ND, Carrick DM, Chander P, Dwyer J, Hamlet MRJ, et al. Biomarkers of chronic inflammation in disease development and prevention: challenges and opportunities. Nat Immunol 2017;18(11):1175-80.

4. Wang RX, Zhou M, Ma HL, Qiao YB, Li QS. The role of chronic inflammation in various diseases and anti-inflammatory therapies containing natural products. Chem Med Chem 2021;16(10):1576-92.

5. Dary C, Hul S, Kim S, Jabbour F. Lectotypification of *Stephania pierrei* (Menispermaceae). Edinb. J. Bot 2015;72:423-28.

6. Waeber C, Moskowitz MA. Migraine as an inflammatory disorder. Neurology 2005;64(10 Suppl 2):S9-S15.

7. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. Alzheimers Dement 2018;4:575–90.

8. Tappayuthpijarn P, Itharat A, Makchuchit S. Acetylcholinesterase inhibitory activity of Thai traditional nootropic remedy and its herbal ingredients. J Med Assoc Thai 2011;94 (Suppl 7):S183-9.

9. Kamonwannasit S, Kamcharoen A. Phytochemical screening and antibacterial activities of tuber extract of *Stephania pierrei* Diels. Chiang Mai J. Sci 2018;45(3):1396-406.

10. Kamonwannasit S, Rupitak Q, Kamcharoen A, Antifungal and antioxidant activities of the extract of *Stephania pierrei* tubers In: Wongwailkhit K, editors. Proceedings of the 4th RSU International Research Conference on Science and Technology, Social Science, and Humanities 2019: RSUSSH 2019; 2019 April 26; Rangsit University: Research Institute, Rangsit University: International Research Conference; 2019. pp.610-615.

11. Bailly C. Cepharanthine: An update of its mode of action, pharmacological properties and medical applications. Phytomedicine 2019;62(152956):1-13.

12. Likhitwitayawuid K, Angerhofer CK, Chai H, Pezzuto JM, Cordell JA, et al. Cytotoxic and antimalarial alkaloids from the tubers of *Stephania pierrei*. J. Nat. Prod 1993;56(9):1468-78.

13. Kamonwannasit S, Nantapong N, Kumkrai P, Luecha P, Kupittayanant S, Chudapongse N. Antibacterial activity of *Aquilaria crassna* leaf extract against *Staphylococcus epidermidis* by disruption of cell wall. Ann Clin Microbiol Antimicrob 2013;12:1-7.

14. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple “test tube” assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem 1987;165(1):215-9.

15. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Communications 1972;46(2):849-54.